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Vasodilator Signals From the Ischemic Myocardium Are Transduced to the Coronary Vascular Wall by Pertussis Toxin-Sensitive G Proteins

A New Experimental Method for the Analysis of the Interaction Between the Myocardium and Coronary Vessels

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OBJECTIVES We sought to detect cross-talk between the beating heart and coronary vascular bed during myocardial ischemia and to test the hypothesis that the cross-talk is mediated by pertussis toxin (PTX)-sensitive G proteins (G_{PTX}) in vessels.

BACKGROUND Coronary flow is closely related to the myocardial metabolic state, indicating the existence of a close interaction between cardiac muscle and coronary vascular beds. Experimental methods for the analysis of the interaction, however, have not been established.

METHODS Coronary detector vessels (DVs) were isolated from rabbit hearts. One end of the vessel was cannulated to a micropipette, and the other end was ligated. After the DV was pressurized (60 cm H_2O), it was gently placed on the myocardium, which was perfused by the left anterior descending coronary artery (LAD) of anesthetized, open-chest dogs (n = 23). The LAD was occluded, and the DV diameter was observed using an intravital microscope with a floating objective system. To evaluate the involvement of G_{PTX}, the DV was pre-incubated with PTX (100 ng/ml).

RESULTS The LAD occlusion of the beating heart produced significant dilation of DVs (241 ± 25 μm) by 10%. The DVs pretreated with PTX (250 ± 27 μm) did not dilate in response to myocardial ischemia. N^{ω}-nitro-L-arginine (100 μmol/l), but not glibenclamide (5 μmol/l), abolished the ischemia-induced DV dilation.

CONCLUSIONS We have established experimental methods for direct analysis of the interaction between the myocardium and coronary microvessels. We conclude that the ischemic myocardium releases transferable vasodilator signals that are transduced by means of the G_{PTX} located in the vascular walls. The nitric oxide pathway is involved in the signal transduction. (J Am Coll Cardiol 2002;39:1859–65) © 2002 by the American College of Cardiology Foundation

Because the cardiac metabolic rate is high due to its continuous beating, the changes in oxygen uptake are mainly determined by coronary flow changes. In fact, previous studies have shown a close correlation between coronary flow and the metabolic state of the heart (1,2). However, because of the lack of adequate methods for evaluating the cross-talk between cardiac muscle and the coronary vascular bed, it has not been well clarified how vasomotor signals from cardiac muscle cells modulate the tone of coronary vessels.

To identify the mechanisms of the regulation of coronary microvascular tone, many studies have been performed. We previously observed the coronary microvascular responses during autoregulation, brief ischemia and metabolic stimulation in vivo (3–5) and demonstrated the importance of adenosine triphosphate-sensitive potassium channels (K_{ATP}) and pertussis toxin (PTX)-sensitive G proteins (G_{PTX}) in the regulatory mechanism. However, vascular responses of the beating heart are determined by many factors, such as myogenic control, shear stress-induced dilation, metabolic factors and neurohormonal control (6,7). For example, under an ischemic condition, the vascular tone is affected not only by myocardial metabolic changes but also by decreases in the intraluminal distending pressure, decreases in shear stress, neurohormonal control and so forth. Therefore, it is difficult to precisely evaluate the vasodilator signals from the myocardium in vivo.

On the other hand, in vitro studies have been performed to investigate the effects of vasoactive factors that could be involved in metabolic vascular control. Adenosine (8) and a decrease in pH (9), which have been suggested to be involved in coronary flow regulation during hypoxia, ischemia and increased metabolic demand of the heart, have been examined in isolated coronary vessels. However, it is difficult to extrapolate the relevance of these vasoactive factors in the metabolic control of the vascular diameter in vivo from the in vitro studies. To investigate the cross-talk between coronary vessels and the myocardium, we have developed a new experimental method in which isolated

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Abbreviations and Acronyms

DV	= detector vessel
G_{PTX}	= pertussis toxin-sensitive G proteins
K_{ATP}	= adenosine triphosphate-sensitive potassium channels
LAD	= left anterior descending coronary artery
L-NNA	= N^{ω}-nitro-L-arginine
LV	= left ventricle
NO	= nitric oxide
PSS	= physiologic saline solution
PTX	= pertussis toxin
SNP	= sodium nitroprusside

detector vessels (DVs) were placed on the beating heart. With this method, the distending pressure of the DVs, the neurohormonal environment around the isolated vessels and the metabolic state of the beating heart can be independently controlled.

Heterotrimeric G proteins are crucial in the transduction of various biologic signals from the outside to the inside of the cell (10–12). We have shown that they are important regulatory modulators in the coronary circulation because they play a pivotal role in the control of the coronary microvascular tone in vivo (4,5). Therefore, we investigated whether the signals released from myocytes under the condition of ischemia are sensitive to PTX.

We have established a new method by which we can separately control the beating heart and coronary vasculature by combining isolated vessels with a beating-heart preparation. Our objectives were to clarify whether the ischemic myocardium releases transferable vasodilator signals and whether the vasodilator signals from the ischemic heart are transduced by means of the G_{PTX} located in the vascular wall. We also investigated the involvement of K_{ATP} and L-arginine–nitric oxide (NO) pathway in the signal transduction.

METHODS

Preparation of DVs. Male Japanese white rabbits (n = 23, weight 2.4 ± 0.1 kg, 10 to 13 weeks old) were anesthetized with an intravenous injection of pentobarbital (50 mg/kg) and heparinized (800 U intravenously) to prevent blood coagulation. After the rabbits were sacrificed by bleeding from the carotid artery, the hearts were quickly excised and immediately immersed in chilled physiologic saline solution (in mmol/l: NaCl 145.0, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.17, NaH_2PO_4 1.2, pyruvate 2.0, calcium disodium EDTA 0.02, 3-[*N*-morpholino]-propanesulfonic acid 3.0 and glucose 5.0), maintained at 0°C and buffered to pH 7.40. A coronary arterial vessel perfusing the left ventricle (LV) (100 to 400 μm) was carefully and gently dissected without excessive force. One end of the vessel was cannulated to a polyethylene micropipette (filled with filtered PSS), the tip of which was tapered (<100 μm), and tied with a monofilament of silk suture thread (diameter 2 μm). The polyethylene micropipette was connected to a pressure reservoir. The other end of the vessel was ligated completely, and the intraluminal pressure was monitored with a strain-gauge transducer (TC0406S01, Baxter Corp.) to detect vascular leaks. Vessels with any leakage were not used for further experiments. The DV was incubated in oxygenated warm PSS (38°C) until use.

Beating-heart preparation. Mongrel dogs of either gender (n = 23, body weight 2.5 to 12.4 kg [mean 7.5 ± 0.5]) were used and prepared as described in our previous in vivo studies (13,14). Briefly, the dogs were anesthetized with alpha-chloralose (60 mg/kg, Wako Chemicals, Osaka, Japan) and artificially ventilated. The right jugular vein was cannulated for administration of the anesthetic agents and fluid infusion. A catheter was introduced into the right carotid artery, and its tip was placed in the ascending aorta for the measurement of aortic pressure with a strain-gauge transducer (TC0406S01, Baxter Corp.). A thoracotomy was performed, and the pericardium was cut to expose the heart surface. The electrocardiogram was monitored throughout the experiment.

The heart rate was kept constant at 100 to 110 beats/min by left atrial pacing after suppression of the sinus node with an injection of formaldehyde (~0.5 ml). Two 24-gauge, stainless-steel needles were inserted horizontally (5 to 7 mm apart) into the mid-myocardium of the LV. Both ends of each needle were fixed to a needle holder held with coil springs. This apparatus limits the excessive perpendicular and horizontal movement of the heart, thereby keeping the region of interest within the observation field.

The main trunk of the left anterior descending coronary artery (LAD) was dissected, and a snare (4-0 silk suture thread) was placed around the LAD for coronary occlusion. In several experiments, a small branch of the LAD distal to the occlusion point was cannulated with a small catheter (outer diameter 0.67 mm), and the distal coronary pressure was measured.

The investigation conformed to the "Guide for the Care and Use of Laboratory Animals," published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the experimental protocols were approved by the institutional Committee for Animal Experiments.

Bioassay method. The incubated DV was gently placed on the LAD perfusion area of the beating LV of the dog. Only one DV was used for each experiment. The DV and the heart surface were kept moist throughout the experiment by continuous superfusion of PSS at a rate of 60 ml/h. The entire superfusion line was continuously warmed by a warm circuit, using a thermostat to keep the superfusate temperature at 37°C on the heart surface. An internal pressure of 60 cm H_2O was applied to the vessels to produce intrinsic tone, and the internal diameter was observed.

Image acquisition system. For visualization of the DV placed on the beating LVs, an intravital microscope system equipped with a floating objective was used. This optical

system was originally developed for visualization of coronary microvessels of the beating-heart surface in vivo in our laboratory (13,14). With this system, it is possible to continuously observe the surface of the beating heart. The microscope objective was a Leitz model PL-fl (×10; numeric aperture of 0.30). An objective lifter was used to carefully adjust the distance between the objective and vessels, so as not to compress the DV.

Epi-illumination by a stroboscopic light source was applied to obtain the image of the DV and measure the internal diameters. A polarizing filter (IGS, Nikon, Tokyo, Japan) was used to minimize reflected light from the surface of the heart. Obtained images were monitored and recorded at 200 frames/s, using a high-speed video camera (model MHS-200, Nac Inc., Tokyo, Japan) with an image intensifier (C 3100, Hamamatsu Photonics, Hamamatsu, Japan). The spatial resolution of this system was 2 μm.

Vascular diameter measurement. The inner diameters of the DVs were measured on a high-resolution monitor screen (C 1846-01, Hamamatsu Photonics) using a video manipulator (C 2117, Hamamatsu Photonics). On the monitor screen, one cursor was set on the vessel wall of interest, and another cursor was set on the nearest point of the other side of the vessel wall. The distance between the two cursors was automatically calculated by the video manipulator, thereby yielding the vascular diameters. Vascular diameters were measured at least three times during the end-diastolic phase. To evaluate the diameter changes for each intervention, the diameters were measured at the same point using the polyethylene micropipette or the tying thread as a reference point.

Experimental protocols. Experiments were performed approximately 1 h after placing the DVs onto the beating hearts, when all monitored variables had become stable and the intrinsic tone of the DVs had developed.

PROTOCOL 1 (N = 10). We investigated whether the ischemic myocardium releases vasoactive signals that are transmitted to DVs. After measurement of the monitored baseline variables, the LAD of the beating heart was completely occluded. Images of the DV were collected at 2, 3, 5 and 10 min after the onset of ischemia. Hemodynamic variables were collected 5 and 10 min after the onset of ischemia.

PROTOCOL 2 (N = 4). We investigated whether the vasoactive signals from the ischemic myocardium are transmitted to DVs by G_{PTX} located in the vascular wall. For this purpose, the DVs were pretreated with PTX (100 ng/ml) for 2 h. Thereafter, the DV was placed on the beating hearts. After measuring the baseline data, the LAD of the beating heart was completely occluded. Vascular images and hemodynamic data were collected in the same fashion as in Protocol 1.

In additional experiments, we tested whether the cross-talk between the ischemic myocardium and the DV involves the NO pathway or K_{ATP} activation, or both, because we have previously shown that microvascular G_{PTX} is functionally linked with those mechanisms (15). For these purposes, we used N^{ω}-nitro-L-arginine (L-NNA; 100 μmol/l; n = 4) to block the NO pathway, and glibenclamide (5 μmol/l; n = 5) to block K_{ATP}. Because these agents are reversible blockers, we superfused them onto the DV after placing it on the myocardium throughout the experiment, unlike PTX experiments. Twenty minutes after the onset of superfusion of these blockers, the LAD was occluded, and the DVs were observed in the same manner as in Protocols 1 and 2. The images of the DVs were obtained 5 min after the ischemia.

In each experiment, ischemia of the myocardium on which the DV was placed was confirmed by the dyskinetic wall motion and cyanotic color changes of the heart. At the end of the experiment, sodium nitroprusside (SNP; 100 μmol/l) was superfused for 5 min to produce maximal dilation.

Drugs. The PTX solution (200 μg/ml, Seikagaku Co., Tokyo, Japan) was freshly diluted with PSS to 100 ng/ml on each experimental day. Glibenclamide (Sigma, St. Louis, Missouri) was dissolved with dimethylsulfoxide at first, and then the target concentration was obtained with PSS. The final concentration of dimethylsulfoxide was 0.005 vol%. L-NNA (Sigma) and SNP (Wako Chemicals) were freshly dissolved in PSS to the desired concentrations.

Data analysis. The aortic pressure (phasic and mean) and distal coronary pressure (mean) were recorded on a Rectigraph (model 8K, San-Ei Sokki, Tokyo, Japan). All variables were expressed as the mean value ± SEM. The responses of the DV diameters to myocardial ischemia were expressed as the percent change in diameter. When the baseline diameter was >90% of the maximal diameter produced by SNP, the vessel was discarded (n = 23). When ventricular fibrillation occurred during the protocol, the experiment was excluded from the data analysis (n = 7). Changes in aortic pressure and vascular diameters were statistically analyzed by using one-way analysis of variance for repeated measures and the Student *t* test for paired samples, with the Bonferroni correction applied to detect the time point when significant changes occurred. At $p < 0.05$, the differences were accepted as significant.

RESULTS

The aortic pressure slightly decreased at 5 min, but not at 10 min, in Protocols 1 and 2 (Table 1). The blood gas and pH were kept within physiologic ranges before ischemia (Table 1). In five cases (3 in Protocol 1, 2 in Protocol 2), we measured the distal coronary pressure of the occlusion site and found that the coronary occlusion produced a significant reduction in the perfusion pressure in both Protocol 1 (before ischemia: 91 ± 12 mm Hg; 5-min ischemia: 23 ± 3 mm Hg; 10-min ischemia: 24 ± 4 mm Hg) and Protocol 2 (before ischemia: 108 ± 10 mm Hg; 5-min ischemia: 31 ± 1 mm Hg; 10-min ischemia: 33 ± 3 mm Hg).

After incubation in a bath of PSS, the DVs were placed on the beating heart. The focusing point for the heart's

Table 1. Aortic Pressure, Blood pH and Blood Gas Data of Dogs in Protocols 1 and 2

	Mean Aortic Pressure (mm Hg)	Blood pH	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)
Protocol 1 (n = 10)				
Before ischemia	103 ± 8	7.40 ± 0.01	99 ± 7	32 ± 2
5-min ischemia	94 ± 7*			
10-min ischemia	98 ± 7			
Protocol 2 (n = 4)				
Before ischemia	113 ± 7	7.43 ± 0.04	107 ± 19	31 ± 2
5-min ischemia	104 ± 8			
10-min ischemia	107 ± 6			

*$p < 0.05$ compared with before ischemia. Data are presented as the mean value ± SD. P_{O_2} = partial pressure of oxygen; P_{CO_2} = partial pressure of carbon dioxide.

surface was lower than that for the DVs, so that the DVs were not compressed by the floating objective system.

Figure 1 shows the responses of the DVs in Protocol 1. The DVs (10 vessels; baseline diameter: 241 ± 25 μm) began to dilate significantly 2 min after the induction of ischemia. The dilation attained its peak at 3 min and continued for 10 min.

In contrast, in Protocol 2, the DVs pretreated with PTX (4 vessels; baseline diameter: 250 ± 27 μm) did not dilate in response to myocardial ischemia (Fig. 2). There were no differences in the dilator responses to SNP (100 μmol/l) between Protocol 1 and Protocol 2 (Fig. 3). Because only DVs were pretreated with PTX, these results indicate that the vasodilator signals from the ischemic myocardium are transduced to the DVs by G_{PTX}, which is located in the vascular wall.

When L-NNA (100 μmol/l) was superfused onto the bioassay system (n = 4; baseline diameter: 208 ± 26 μm), the DV did not dilate but actually constricted in response to myocardial ischemia (−18.5 ± 7.4% at 5 min after LAD occlusion; $p < 0.05$ vs. Protocol 1). Sodium nitroprusside dilated the DVs by 13.5 ± 4.5%.

When glibenclamide (5 μmol/l) was superfused onto the bioassay system (n = 5; baseline diameter: 194 ± 24 μm),

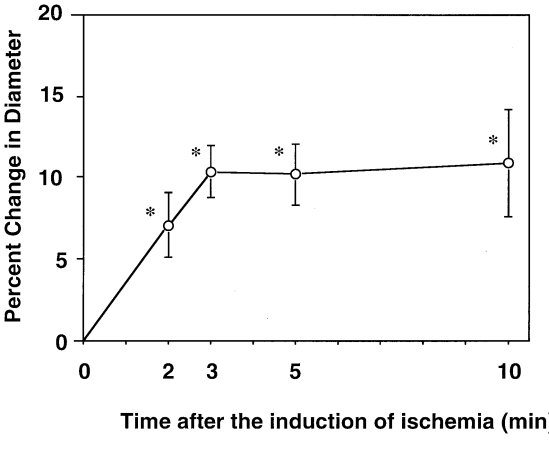

Figure 1. The detector vessel responses during myocardial ischemia in Protocol 1. Significant dilation was observed at 2, 3, 5 and 10 min after the induction of ischemia. *$p < 0.05$ compared with baseline.

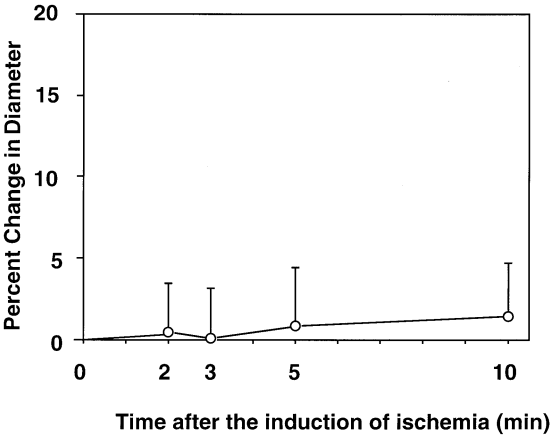

Figure 2. The detector vessel (DV) responses during myocardial ischemia in Protocol 2. In contrast to Protocol 1, no significant dilation was observed during ischemia, when the DVs were pretreated with pertussis toxin (100 μg/ml).

the DVs still dilated in response to myocardial ischemia (6.5 ± 3.8%; p = NS vs. Protocol 1). Sodium nitroprusside dilated the DVs by 17.9 ± 1.8%.

DISCUSSION

In the present study, we have established a new experimental method for analyzing the interaction between cardiac muscle and coronary arterial vessels. With this method, we have found the following: 1) the ischemic heart releases vasoactive signals that cause vascular dilation. The vessels begin to dilate within 2 min, and the maximal dilation continues for 10 min. 2) The vasodilator signals from the ischemic heart are transduced by G_{PTX}, which is located in the vascular wall. 3) Production of NO is involved in this signal transduction.

Methodologic consideration. This is a unique method combining isolated vessels with a beating-heart preparation for analyzing the interaction between the heart and the

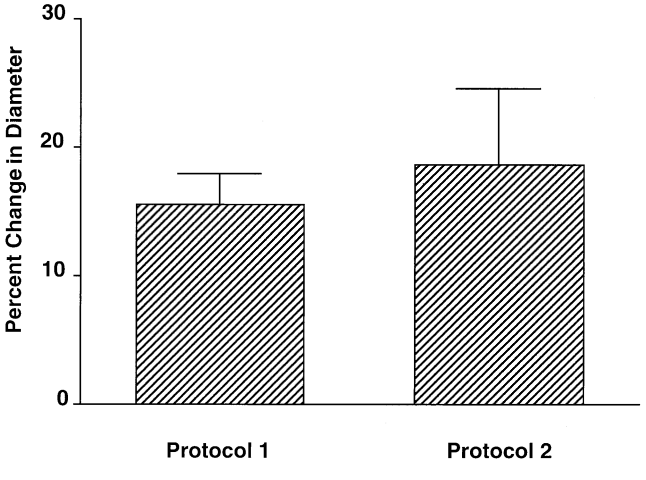

Figure 3. Column graphs showing the detector vessel responses to superfusion of sodium nitroprusside (SNP) (100 μmol/l). In both groups, the vessels were significantly dilated. There was no difference in the magnitude of SNP-induced dilation between Protocols 1 and 2.

coronary vasculature. The detector coronary vessels were placed directly on the beating heart, and time-matching, time-dependent observation of the DVs could be performed. We believe that the dilation of the DVs caused by myocardial ischemia was not an artifact, because the dilation was specifically blocked by PTX, and the dilator capacity of the DVs for SNP was well preserved.

In the present bioassay method, the beating heart and the DV could be controlled independently of each other. In these experiments, the intravascular distending pressure was set at 60 cm H_2O throughout the experiment. In the in vivo observations of the coronary circulation, coronary occlusion produced a decrease in the intravascular pressure along with the myocardial ischemic condition, both of which affect the vascular tone. The vasorelaxation of vessels >200 μm during ischemia cannot be detected in an in vivo preparation, possibly because the vasorelaxation is balanced by vascular collapse caused by the decrease in intravascular pressure (3). The present method is superior in terms of its precise detection of vasodilator signals from the ischemic myocardium. Furthermore, pharmacologic intervention can also be separately applied to the myocardium and DVs. We applied PTX only to the DVs in the present experiment.

Another advantage of the present method is the minimization of neurohormonal effects. Because the DVs are not innervated and the effect of the beating heart's hormonal changes on the DV would be minimal, we can precisely assess the dilatory metabolic signal released from the beating heart.

With this method, we could detect, with sensitivity, the dilator signals from the ischemic myocardium, as the dilation occurred as quickly as 2 min after the induction of myocardial ischemia. Our previous study, however, has shown that microvascular dilation takes place 30 s after the onset of coronary occlusion (16). The time lag of 1.5 min may have been caused by the barrier function of the epicardium between the surface of the heart and the DV.

There is an earlier study that focuses on the interaction between cardiomyocytes and coronary microvessels. Tiefenbacher et al. (17) attempted to evaluate vasoactive signals from myocytes to reconcile the paradox that alpha-adrenergic activation in vivo causes constriction of coronary arterioles, whereas an alpha-agonist does not constrict isolated arterioles. For this purpose, they put a supernatant of enzymatically resolved myocytes onto the isolated coronary vessels and demonstrated that some vasoconstrictive factors are released from myocytes upon alpha-stimulation. Compared with the method by Tiefenbacher et al., the present method is more physiologic because the signals we evaluated were derived from beating hearts, not isolated myocytes. Therefore, interaction between the myocardium and coronary vessels that takes place during physiologic and pathophysiologic phenomena, such as ischemia, can be investigated. It is also possible to follow the time course of the interaction by using the present method.

Relatively large microvessels (~250 μm) were used for DVs. The smaller the microvascular size, the larger the amount of vascular resistance these microvessels bear. However, smaller microvessels have thinner vascular walls, which are harder to detect on the beating heart. As a previous study has shown that microvessels >200 μm bear 25% of the total coronary resistance (18), we believe that the present findings are still relevant to the regulation of coronary resistance.

PTX-sensitive mechanisms during ischemia. In a previous study, we showed that vasodilation caused by a reduction in perfusion pressure in the in vivo condition was totally abolished by PTX application (4), indicating the importance of G_{PTX} in the signal transduction for microvascular dilation in response to low perfusion. These findings are consistent with the present data. That study, however, did not answer the question of whether hypoperfusion-induced vasomotion is mediated by G_{PTX} located in the microvascular wall or in the myocardium, because PTX was superfused onto the heart surface, leading to blockade of G_{PTX} both in coronary microvessels and the myocardium around them. G_{PTX} is known to exist not only in vessels but also in the myocardium (19,20). In contrast, in the present study, because only vessels were pretreated with PTX, which irreversibly blocks G_{PTX}, we can now conclude that vasomotor signals from the ischemic myocardium activate G_{PTX} located in the vascular wall.

To identify the mechanisms distal to G_{PTX} activation, we investigated the effect of blockade of the NO pathway and K_{ATP} on the microvascular dilation induced by myocardial ischemia, because we have previously shown that G_{PTX} is functionally linked with those two downstream mechanisms in coronary microvessels (15).

L-NNA completely abolished the dilation of the DVs, whereas glibenclamide was without effect. These results indicate that the L-arginine–NO pathway is substantially involved in the cross-talk between the ischemic myocardium and the coronary microvessels. Kitakaze et al. (21) have shown that NO plays an important role in regulating coronary flow during myocardial ischemia.

We have shown in a previous study (15) that the mechanisms of microvascular dilation in response to G_{PTX} activation are dependent on the vessel size in vivo—that is, K_{ATP} activation is the main mechanism in small microvessels <130 μm, whereas both K_{ATP} and the NO pathway contribute to the dilation in larger microvessels (>130 μm). Because the DVs in the present study were larger than the microvessels we used in the previous in vivo study, it is conceivable that the NO pathway is the main effector of G_{PTX} activation in large microvessels (~250 μm). It is possible that, if we had used smaller DVs, we would have observed the contribution of K_{ATP} in DV dilation.

Myocardial ischemia produced DV constriction in the presence of L-NNA, whereas PTX only offset the dilation. These interesting phenomena suggest that the ischemic myocardium releases vasoconstrictor signals that are PTX-

sensitive, as well as dilator signals. The mechanisms of the vasoconstriction require further investigation.

We cannot conclude which upstream mechanisms activate vascular G_{PTX} during myocardial ischemia. There are several possible mediators that produce the activation of G_{PTX} and vasodilation.

Ohno et al. (22) have previously shown that shear stress activates G_{PTX} in vascular endothelial cells and that this G_{PTX} activation partly mediates NO production. However, in the present study, the DV was ligated, and it was a stop-flow in vitro preparation. Accordingly, it is unlikely that the activation of G_{PTX} that we observed was caused by shear stress.

Adenosine, which is produced during myocardial ischemia, might activate vascular G_{PTX}. Hein and Kuo (8) have recently shown that adenosine activates G_{PTX} on the vascular endothelium, leading to the activation of K_{ATP} and NO production, whereas the direct dilator effect of this nucleoside on vascular smooth muscle is not mediated by G_{PTX} activation. We did not test the effect of adenosine blockers in the present study, because the DVs we used were too large to respond to adenosine (23).

Some studies have addressed the role of adenosine in microvascular regulation during myocardial ischemia. Duncker et al. (24) have shown that an adenosine blocker causes a slight downward shift in the coronary pressure–flow curve below the autoregulatory breakpoint in exercised, awake dogs. That study suggested the involvement of adenosine in vasomotion under the condition of ischemia, but such an involvement, if any, is not substantial. Moreover, we have previously shown that neither the adenosine receptor blocker nor adenosine deaminase inhibited the coronary microvascular dilation at all during reductions in perfusion pressure (25).

Changes in the pH of the myocardium and interstitium are among the possible mechanisms. Myocardial ischemia rapidly produces cardiac interstitial acidosis (26). Extravascular acidosis is known to produce coronary microvascular dilation through the activation of K_{ATP} (27). Ishizaka et al. (9) have shown that G_{PTX} activation is responsible for acidosis-induced vasorelaxation. Although G proteins generally transduce the signal of agonist-bound receptors to intracellular effectors, recent evidence has shown that G-protein activation does not necessarily require receptor occupation (28). Actually, Ishizaka et al. (9) have observed that purified G proteins reconstituted in liposomes are directly activated by acidosis.

In the present experiments, the superfusate buffer solution that was applied to keep the vessels moist and warm may have attenuated the reduction in pH during ischemia. However, as the DVs were in direct contact with the myocardium, protons from the myocardial tissue could possibly activate the dilator signal transduction in the DVs. Because acidosis-induced dilation of coronary microvessels is an important mechanism in metabolic coronary flow regulation, it is interesting to speculate that protons mediate the cross-talk between the myocardium and coronary vascular bed.

Conclusions. We have developed a new experimental model for evaluating the cross-talk between the myocardium and coronary microvessels. The present study has clearly shown that the ischemic myocardium releases transferable factors that dilate the coronary microvessels and that G_{PTX} plays a crucial role in the cross-talk. The L-arginine–NO pathway is involved in the signal transduction. The precise upstream and downstream mechanisms remain to be determined.

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